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Factors limiting early establishment of the Mediterranean grassland species *Brachypodium retusum* at disturbed sites

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Abstract

In Mediterranean dry grasslands, water availability and grazing strongly influence plant establishment. Although higher water availability and lower grazing pressure usually increase seedling recruitment in the beginning, higher competition may reduce seedling survival. In our study, we analysed this trade-off for the dominant species of Western Mediterranean steppes *Brachypodium retusum* which hardly recolonises restoration sites degraded by soil disturbance such as arable use.

A split-plot experiment was set up on an ex-arable site in the “La Crau” steppe in order to test the effects of initial watering and sheep grazing on seedling recruitment, survival and early growth during two years. The watering treatment was applied in the first autumn and spring doubling the usual precipitation during this period and grazing was tested using exclosures. Survival and growth of seedlings established from field-sown seeds were compared to those of transplanted seedlings pre-grown in a growth chamber.

Watering did not affect germination whereas the grazing effect was negative. Initial watering had a positive effect on survival only in grazed plots suggesting a compensatory effect under grazing disturbance. Significant main effects of watering on early growth disappeared in the second season. Grazing significantly reduced most measured growth traits whereas the effect on survival was only marginally significant. Planted seedlings (49%) survived better than seedlings emerged from field-sown seeds (36%). In conclusion, initial fencing increases establishment success of *B. retusum* in ecological restoration. Initial watering may help to mitigate negative grazing effects when fencing is not possible.

Keywords: Dry grassland; Plant–environment interactions; Poaceae; Population dynamics

Introduction

European semi-natural grasslands are under constant decline and belong to the most threatened habitats today (Poschlod & WallisDeVries 2002; Pywell et al., 2007). Grasslands have been frequently converted to arable fields because

improved drainage and irrigation techniques allow nowadays crop production at sites previously limited to extensive grassland use (Bischoff, Warthemann, & Klotz 2009; Tatin, Wolff, Boutin, Colliot, & Dutoit 2013). Under Mediterranean climate, strong summer drought limits standing biomass, and the combination of grazing and climate effects has resulted in particularly species-rich grasslands also called pseudo-steppes dominated by *Brachypodium retusum* (San Miguel 2008; Blondel, Aronson, Bodiou, & Boeuf 2010). Fire may

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also have contributed to the development of such pseudo-steppes but its role is less well understood (San Miguel 2008; Vidaller, Dutoit, Ramone, & Bischoff 2019). Ecological restoration of Mediterranean grasslands based on spontaneous succession is a slow process. In particular, characteristic species that are limited to this type of grasslands slowly establish or even remain absent (Blondel & Aronson 1999; Römermann, Dutoit, Poschlod, & Buisson 2005; Coiffait-Gombault, Buisson, & Dutoit 2012).

Water availability is well-known to be a limiting factor of plant establishment (Noy-Meir 1973; Ronnenberg, Hensen, & Wesche 2011), which may affect ecological restoration. Drought stress reduces germination and increases seedling mortality (Bochet, García-Fayos, Alborch, & Tormo 2007; Thomas, Morris, Auld, & Haigh 2010). However, an increase in water availability also increases competition for light and other resources (Kadmon 1995). In *B. retusum* grasslands of Southern France, higher soil moisture favours undesired ruderal species such as the high-growing grass *Brachypodium phoenicoides* that outcompete typical pseudo-steppe species (Masson, Mesléard, & Dutoit 2014).

Mediterranean dry grasslands depend on human land management, in particular on grazing (San Miguel 2008; Apostolova, Dengler, Di Pietro, Gavilán, & Tsiripidis 2014). Land abandonment results in shrub encroachment which is together with land use intensification the major threat to this type of ecosystem (San Miguel 2008). Grazing animals remove biomass reducing above-ground competition and create gaps by trampling, both shaping plant communities and favouring the co-existence of different species (Bullock, Pywell, Burke, & Walker 2001; Drobnik, Römermann, Bernhardt-Römermann, & Poschlod 2011; Coiffait-Gombault et al. 2012). Thus, grazing may have positive effects on seedling establishment (Bullock et al. 2001). However, grazing is also known to increase seedling mortality, thereby hampering establishment (Buisson, Corcket, & Dutoit 2015). Although showing a relatively low palatability, young seedlings of *B. retusum* are negatively affected by grazing (Buisson et al. 2015; Vidaller, Dutoit, Ibrahim, Hanslin, & Bischoff 2018).

B. retusum (Pers.) P. Beauv., (ramose false brome, Poaceae), is the dominant perennial grass species of dry grasslands in the Western Mediterranean basin but also occurs in shrublands (matorral, garrigue) and open woodlands (Caturla, Raventós, Guàrdia, & Vallejo 2000; Catalan, López-Álvarez, Díaz-Pérez, Sancho, & López-Herránz 2015). The rhizomes of this perennial grass form a dense network close to the soil surface allowing resprouting after destruction of above-ground organs (Caturla et al. 2000). Clonal propagation is common but slow. *B. retusum* usually flowers from April to July and seeds germinate after autumn rainfall or during early spring when humidity is sufficient (Vidaller et al. 2018). The species is still abundant in the Western Mediterranean but *B. retusum* dominated grasslands (Thero-Brachypodietaea) are under decline and protected by the EU habitats directive (San Miguel 2008). Due to its high cover

and numerous interactions with other species of the community (Saiz & Alados 2011), successful restoration of plant community structure of such grasslands depends on the re-establishment of *B. retusum*. In our study area, the “La Crau” nature reserve in South-Eastern France, *B. retusum* was not able to re-colonise restoration sites within thirty years although pristine dry grasslands with a high population density of the species occur adjacent to these sites (Coiffait-Gombault et al. 2012). Apart from a lack of seeds due to high sterility of fruits (Vidaller et al. 2018) or dispersal limitation (Bischoff 2000), conditions of establishment after arrival of seeds may represent a strong environmental filter (O’Connor 1991; Nuttle 2007; Pywell et al. 2007). We hypothesize that water availability and grazing are key factors limiting seedling establishment. We further hypothesize that initial watering has a positive effect whereas initial grazing has a negative effect on seedling recruitment. We were particularly interested in the interaction of both factors since the joint effect of water stress and grazing on seedling establishment has rarely been tested so far (Ibáñez & Schupp 2001; Soliveres et al., 2011).

We present results of a two-year full-factorial field experiment manipulating water availability and grazing on an ex-arable field converted to traditional dry grassland management in 1985 (Römermann et al. 2005; Buisson, Dutoit, Torre, Römermann, & Poschlod 2006). Since germination and initial seedling growth are particularly critical steps in plant establishment (Bochet et al. 2007; Thomas et al. 2010), we tested effects on seedlings grown from field-sown seeds and on seedlings pre-grown under greenhouse conditions. We specifically asked the following questions: (1) What is the effect of initial watering and grazing on *B. retusum* field germination? (2) What is the effect of initial watering and grazing on the survival and growth of field-sown and transplanted *B. retusum* seedlings? (3) Is there an interaction between the effects of watering and grazing on seedling establishment?

Materials and methods

Study area

The study site is part of the “La Crau” Nature reserve in South-Eastern France. The area is characterised by hot/dry summers and mild/humid winters with an annual precipitation of 517 mm (1980–2010) and a mean temperature of 15.3 °C (see Appendix A: Fig. 1 in Supplementary material). The soils of the study area are decalcified Red Mediterranean Soils (Haplic cambisol) with a mean pH of 6.8. The sites are grazed by itinerant sheep from February to June.

The experiment was set up on a restoration site in “Peau de Meau” (43°34’09 N/4°49’55 E). The pseudo-steppe was converted to arable use in 1960 to grow melons and cereals until 1985 (Buisson et al. 2006). Afterwards, management returned to extensive sheep grazing in the same way as the surrounding pristine grasslands. These surrounding reference sites

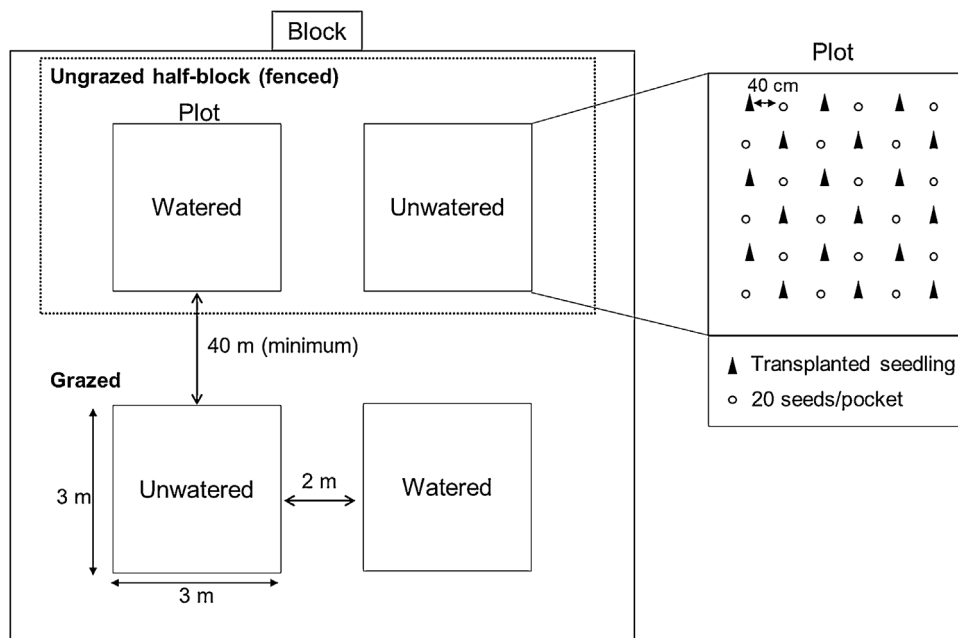


Fig. 1. Position of plots and associated treatments within a block of a field experiment on *B. retusum* seedling establishment in Mediterranean restoration grassland.

belong to the Mediterranean Thero-Brachypodieta habitat “Pseudo-steppe with grasses and annuals” (EUNIS habitat E1.311, Natura 2000 habitat type 6220, San Miguel 2008). Reference and restoration sites of the Crau nature reserve are characterised by a high stone cover preventing the development of dense grassland swards. Although management was the same as on reference sites (mean grazing intensity of 567 sheep ha⁻¹ season⁻¹), *B. retusum* but also several other typical species such as *Asphodelus ayardii*, *Stipa capillata* and *Thymus vulgaris* have not re-occurred on the restoration site. Instead, mesophilic and ruderal species such as *Avena barbata*, *Avenula bromoides*, *Bromus hordeaceus*, *Calamintha nepeta*, *Diploaxis tenuifolia*, *Lobularia maritima*, are more common (Buisson et al. 2015). The species richness of the restoration site is lower than that of reference sites.

Experimental design

In autumn 2016, we set up a split-plot experiment testing the effects of initial watering, grazing and its interaction on germination percentage, seedling survival and growth for (i) transplanted seedlings and (ii) seedlings emerged in the field (Fig. 1). Blocks ($n=5$, distance = 50 m) were split into two parts (half-blocks) of which one was fenced to prevent sheep grazing. The two watering treatments (watering, no watering) were applied to two plots of 9 m² within each half-block (split-plot design) in order to test the combined effects of watering and grazing (20 plots in total). A distance of 40 m between half-blocks within blocks was chosen to avoid any influence of enclosures on grazed plots since grazing is

often much stronger close to enclosures (Saatkamp, Henry, & Dutoit 2018). The position of water treatments was randomised within half-blocks and that of grazing treatment was randomised within blocks.

Seeds from inflorescences of forty 1 m² *B. retusum* patches (patch distance was at least 10 m to guarantee that patches belonged to different individuals) were collected in each of two local populations in the previous year. The seeds were pooled for the following experiments. In each plot, 18 seedlings previously germinated and grown for 2 months in peat plugs (4 cm × 4 cm) in a growth chamber (temperature 15/20 °C, 12/12 h, night/day) were transplanted in October 2016. The average germination in peat plugs was 69%. At the same time, 20 seeds were sown to each of 18 pockets between transplanted seedlings (Fig. 1). Transplanted seedlings and one emerging seedling per pocket were marked for continuous survival and growth measurements. Seeds and seedlings were planted into the natural vegetation and no weeding was applied.

In autumn 2016 (late October to early December) and in spring 2017 (early April to early July), plots of the initial watering treatment were irrigated every seven to fourteen days in order to add 60 l/m² of water per month. Thus, irrigation doubled the average monthly precipitation during this period that both treatments and controls received. The irrigation treatment was interrupted in winter and early spring since soils were water-saturated. The distance between plots within half-blocks was 2 m limiting water flow from irrigated to control plots. The grazing enclosures were set up using fences with stretched, non-electrified nets (1 m high).

Data collection

Individuals were labelled and counted from December 2016 to May 2018 to obtain germination (until May 2017) and survival percentages. Growth traits were measured three times at peak biomass before summer drought in 2017 (July) and 2018 (May) and in early winter 2017 (December) after autumn regrowth. The growth measurements included plant height, diameter, tiller number, leaf number and the length of the longest leaf to characterise plant performance. Plant height was measured from the ground to the top of the shoot which is the insertion point of the highest leaf in vegetative shoots and the top of the inflorescence in flowering or fruiting plants. We calculated cumulative leaf length (leaf length \times number of leaves) and cumulative tiller length as an aboveground biomass estimate (plant height \times number of tillers).

Data analysis

Linear models were run to analyse the effects of watering (yes/no), grazing (yes/no) and planting type (field-sown/greenhouse-sown). The full model included the three factors, all two-way interactions, the three-way interaction watering \times grazing \times planting type and block and was applied to survival and growth traits. In order to consider the split-plot design of the experiment, grazing was tested against the grazing \times block interaction. Watering and the watering \times grazing interaction were tested against the grazing \times watering \times block interaction. Finally, planting type, block and all interactions with planting type were tested against the model residuals. Data analyses for growth traits were performed separately for each survey date. A repeated measures analysis based on this model was calculated for survival since this variable was measured more frequently than growth. A reduced model only including grazing, watering, their interaction and block was applied to germination data. As in the full model, grazing was tested against the grazing \times block interaction. Watering, the watering \times grazing interaction and block were, however, tested against the model residuals. When necessary $\log_{10}(x+1)$ transformations (height, tiller number, cumulative leaf length and cumulative tiller length) and $\arcsin(\sqrt{x/100})$ transformations (germination and survival) were used to comply with assumptions of linear models (normality, homoscedasticity). A Tukey HSD posthoc test was calculated to analyse differences between treatment levels if treatment main effects or interactions were significant.

For growth traits, the effect size of grazing, watering, planting type, and of their interactions was calculated as $100 \times \text{sum of squares (factor)}/\text{total sum of squares}$. All statistical analyses were run in R (R, version 3.3.1, [R Development Core Team, 2013](#)).

Table 1. Effects of initial watering, grazing and seedling type ("type": transplanted versus field-sown) on *B. retusus* germination (only field-sown) and growth (2017 and 2018). T \times W = type \times watering interaction, T \times G = type \times grazing interaction, W \times G = watering \times grazing interaction, T \times W \times G = type \times watering \times grazing interaction. ANOVA *F*-values, significance levels: $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS not significant. Factor effect: \downarrow (decrease) \uparrow (increase).

Date	Factors	df	Germination	Leaf number	Leaf length	Plant height	Tiller number	Cumulative leaf length	Cumulative tiller length	Plant diameter
2017	Type	1	-	4.650	10.650 **	33.326 ***	34.278 ***	7.417 *	39.259 ***	126.101 ***
	Watering	1	1.106 NS	0.607 NS	0.020 NS	1.498 NS	4.649 * \uparrow	1.345 NS	0.681 NS	7.098 ** \uparrow
	Grazing	1	10.540 * \downarrow	0.313 NS	3.821 \downarrow	10.557 ** \downarrow	16.291 ** \uparrow	0.573 NS	0.465 NS	13.222 ** \uparrow
	T \times W	1	-	0.257 NS	0.005 NS	0.065 NS	2.381 NS	0.051 NS	0.959 NS	0.246 NS
	T \times G	1	-	0.977 NS	1.574 NS	1.278 NS	0.023 NS	1.141 NS	0.484 NS	2.181 NS
	W \times G	1	0.478 NS	0.006 NS	0.542 NS	0.713 NS	0.207 NS	0.588 NS	0.032 NS	1.062 NS
	T \times W \times G	1	-	5.523 *	3.804	11.354 **	7.116 *	5.733 *	10.157 **	0.153 NS
2018	Type	1	-	1.605 NS	12.836 *	24.480 ***	7.387 *	7.183 *	18.079 **	20.008 ***
	Watering	1	-	0.430 NS	0.584 NS	0.002 NS	0.289 NS	1.293 NS	0.273 NS	0.887 NS
	Grazing	1	-	2.577 NS	19.594 *** \downarrow	28.501 *** \downarrow	1.506 NS	11.100 ** \downarrow	12.621 *** \downarrow	1.482 NS
	T \times W	1	-	0.175 NS	0.813 NS	0.063 NS	0.002 NS	0.687 NS	0.002 NS	0.303 NS
	T \times G	1	-	1.157 NS	0.298 NS	0.041 NS	1.034 NS	1.233 NS	0.696 NS	0.789 NS
	W \times G	1	-	0.076 NS	0.864 NS	1.068 NS	1.103 NS	0.207 NS	1.611 NS	0.014 NS
	T \times W \times G	1	-	2.241 NS	0.537 NS	0.144 NS	0.180 NS	1.308 NS	0.042 NS	0.291 NS

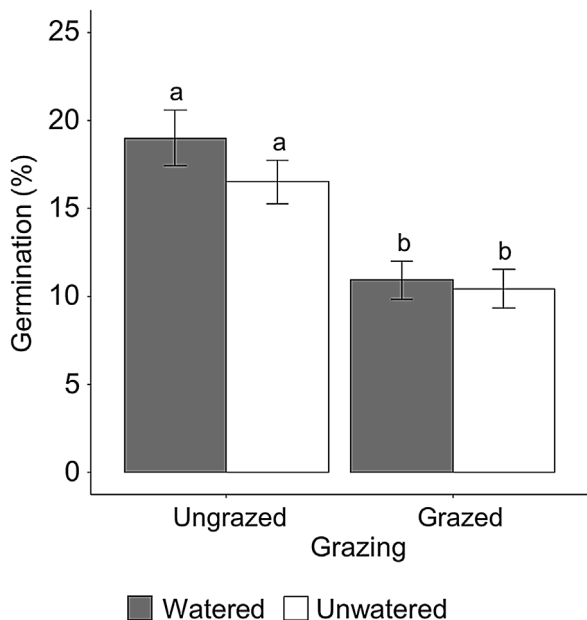


Fig. 2. Effects of initial watering and grazing on final germination of *Brachypodium retusum* (mean values \pm SE).

Results

Field germination ranged from $10.5\% \pm 1.03$ (unwatered and grazed plots) to $19\% \pm 1.56$ (watered and ungrazed plots). Grazing had a significantly negative effect on germination (Table 1, Fig. 2). The effect of watering and the grazing \times watering interaction were not significant.

Watering had no effect on survival, whereas the effect of grazing was marginally significant, with higher survival in ungrazed plots (see Appendix A: Table 1 in Supplementary material, Fig. 3). The grazing effect was stronger in the first than in the second season. A significant grazing \times watering interaction indicated that the watering effect depended on

grazing. A positive effect of watering was only apparent in grazed plots. The significant grazing \times watering \times date interaction showed that this grazing-dependent watering effect also changed with time (see Appendix A: Table 1 in Supplementary material). Field-sown seedlings showed significantly lower survival than planted seedlings, an effect that became stronger during the study period (see Appendix A: Table 1 in Supplementary material, Fig. 3).

The watering effect on plant growth was not significant at the end of the study period (Table 1, Figs. 4 and 5). However, watering significantly increased tiller number, cumulative tiller length and plant diameter in the first season (Table 1; see Appendix A: Table 2 in Supplementary material). Grazing significantly reduced leaf length, plant height, cumulative leaf length and cumulative tiller length (Table 1, Figs. 4C and D, 5). It increased tiller number and plant diameter in the first season whereas this effect was not significant any more in the second season (see Appendix A: Table 2 in Supplementary material, Fig. 4A and B). Significant interactions (watering \times grazing and watering \times grazing \times seedling type) were only found in the first season and disappeared later on. Thus, the growth measurements do not confirm the grazing-dependent increase in survival. Transplanted seedlings showed a significantly higher growth in nearly all measured traits than field-sown seedlings. Only leaf number was not different between seedling types.

Effect size of watering was always lower than that of grazing (Fig. 6). Watering explained between 0.004% (leaf length 2017) and 1.43% (plant diameter 2018, grazing between 0.73% (cumulative tiller length 2017) and 40.85% (tiller number 2018) of the total variation. The grazing \times watering interaction accounted for 0.02% (leaf number 2017) to 6.84% (cumulative leaf length 2018). The effect size of watering decreased from the first to the second season whereas the effect size of grazing increased.

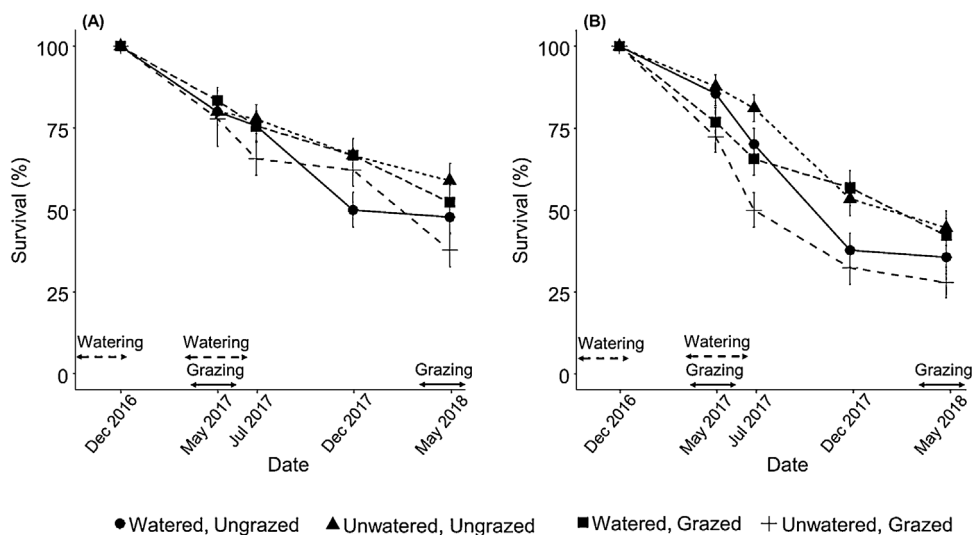


Fig. 3. Effects of initial watering and grazing on the survival of transplanted (A) and field-sown seedlings (B) of *B. retusum* (mean values \pm SE).

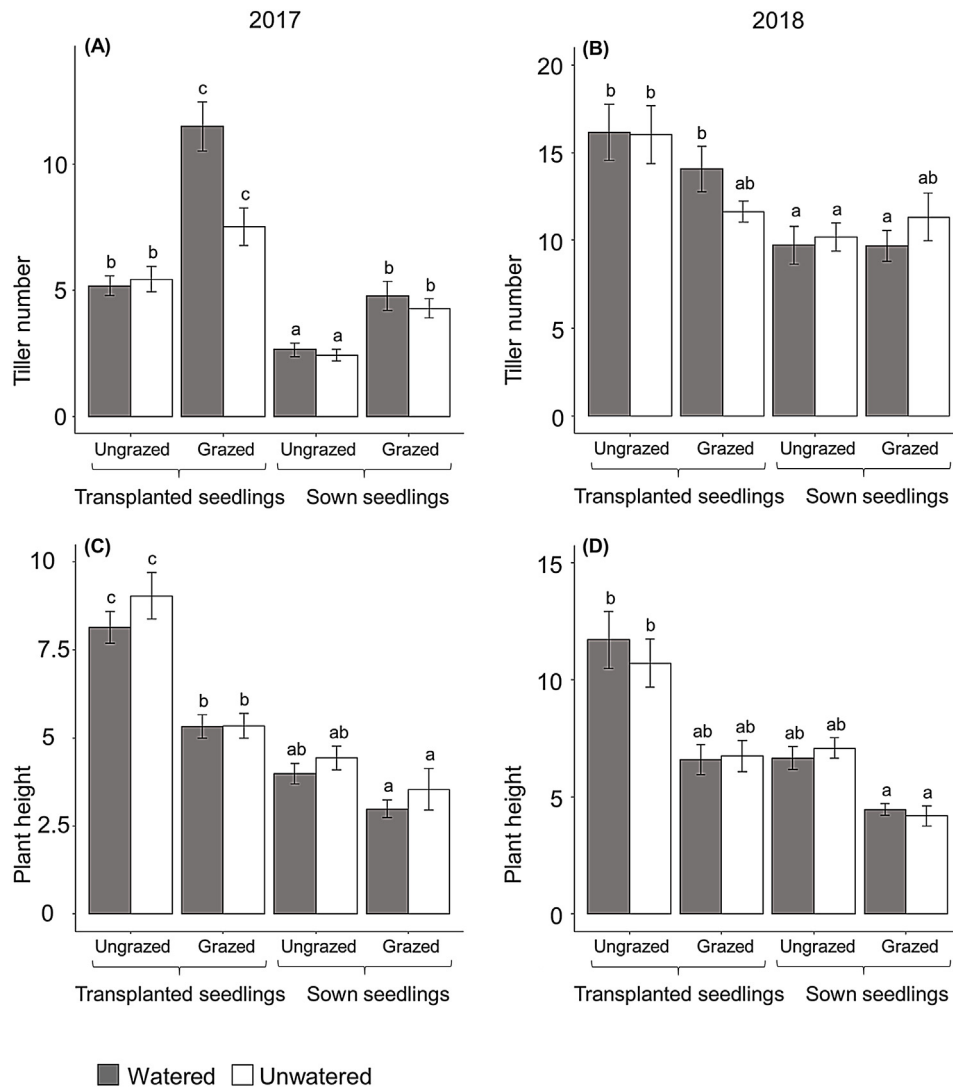


Fig. 4. Effects of initial watering and grazing on field-sown and transplanted *B. retusum* seedlings (mean values \pm SE) at the end of the first grazing period (left side of the panel, 2017) and at the end of the study period (right side of the panel, 2018). (A, B): Tiller number; (C, D): plant height. Different lower case letters indicate significant differences ($P < 0.05$).

Discussion

Both initial watering and grazing were found to affect the establishment of *B. retusum* seedlings in Mediterranean grasslands, 30 years after conversion from intermediate arable use. Initial watering increased performance in early traits but the positive watering effect disappeared already in the second season. Grazing significantly reduced germination and most measured growth traits. A significant watering-by-grazing interaction was found for final survival: Watering increased survival only in grazed plots but not in ungrazed plots.

Field germination

In our study, watering had no significant effect on the germination of *B. retusum*. This result confirmed a previ-

ous growth chamber experiment showing that already at low to moderate moisture levels the response to water addition is not significant (Vidaller et al. 2018). However, it does not correspond to other growth chamber studies in dry or Mediterranean ecosystems showing a very sensitive germination response of *B. retusum* to soil moisture (Bochet et al. 2007; Thomas et al. 2010). Additionally, in several other species pre-emergence mortality between germination and field emergence increases with drought resulting in a stronger drought effect on seedling emergence than on germination (Tobe, Zhang, & Omasa 2005). Our field germination data correspond to seedling emergence since we do not know how many seedlings died before reaching the soil surface. The typical Mediterranean autumn and early-spring rainfall may have been sufficient to avoid seedling mortality in the soil explaining the absence of watering effects on field germination in our study.

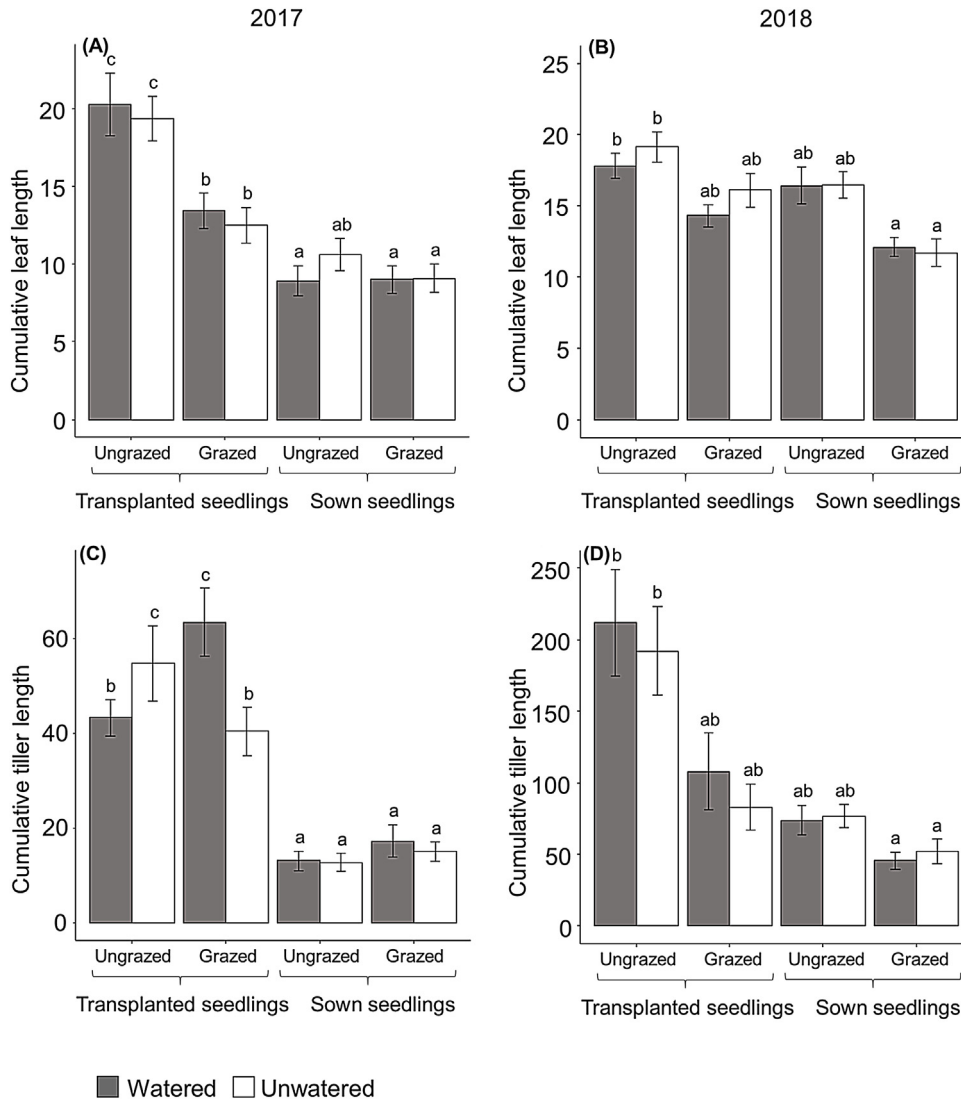


Fig. 5. Effects of initial watering and grazing on field-sown and transplanted *B. retusum* seedlings (mean values \pm SE) at the end of the first grazing period (left side of the panel, 2017) and at the end of the study period (right side of the panel, 2018). (A, B): Cumulative leaf length; (C, D): cumulative tiller length. Different lower case letters indicate significant differences ($P < 0.05$).

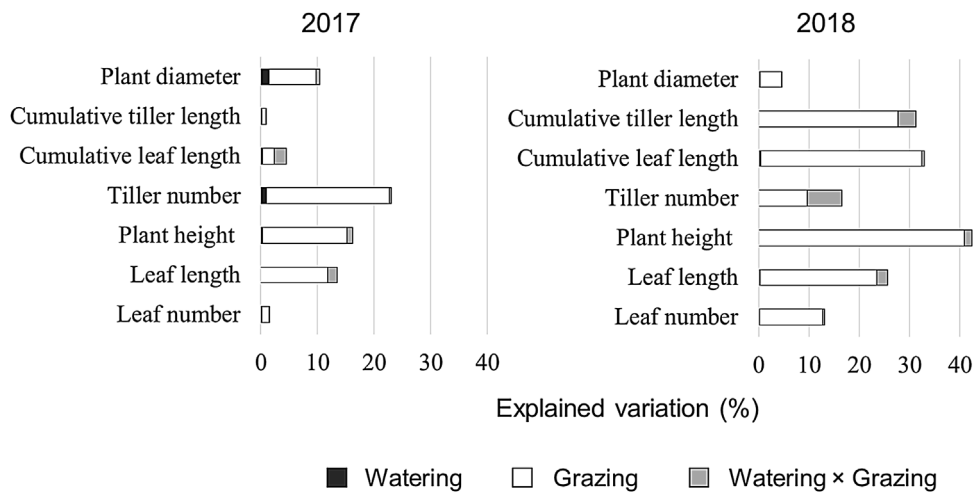


Fig. 6. Relative contribution of watering, grazing and their interaction to total variation (2017 and 2018).

Grazing had a negative effect on field germination. Gap creation by trampling livestock increases germination (Bullock et al. 2001) but mechanical damage by trampling, urine and faeces excretion may have the opposite effect (Bilotta, Brazier, & Haygarth 2007). Trampling is known to damage seeds or emerging coleoptiles (James, Svejcar, & Rinella 2011; Rother, Jordano, Rodrigues, & Pizo 2013). Dung pads may reduce light required by many seeds to germinate (Milotić & Hoffmann 2016). Furthermore, grazing sheep may have damaged seedlings before recording germination in our study.

Survival and growth

The main watering effect on seedling survival was not significant neither in field-sown seedlings nor in seedling pre-grown in a greenhouse confirming that the species is well adapted to drought (Vidaller et al. 2018). *B. retusum* is able to store water in rhizomes and respond fast to rainfall (Clary, Savé, Biel, & Herralde 2004). However, the watering effect depended on grazing and the watering \times grazing interaction was also significantly affected by observation date. Watering had a positive effect in grazed but not in ungrazed plots. This effect was stronger during and after the grazing season which is a particularly stressful period for seedlings. Watering allowed a higher tolerance to grazing whereas watering may increase competition to ruderal species when not stressed by grazing. In similar Mediterranean dry grasslands, higher soil moisture favoured ruderal species such as the high-growing grass *B. phoenicoides* outcompeting typical pseudo-steppe species (Masson et al. 2014). Water addition to ungrazed plots may also increase soil pathogen infestation, reducing plant performance and influencing interspecific competition (Van der Putten & Peters 1997; Van der Putten, Vet, Harvey, & Wäckers 2001).

The watering treatment had a positive effect on growth in the first season. In ecosystems with seasonal aridity, water is a limiting factor for growth (Noy-Meir 1973) mainly due to a trade-off between drought tolerance and growth capacity (Pérez-Ramos, Volaire, Fattet, Blanchard, & Roumet 2013). Very young *B. retusum* plants allocate resources to tillering and leaf growth. Later, tillers elongate and older leaves die (Vidaller et al., 2018). Watering increased leaf growth and tillering in the beginning and tiller growth up to seven months after treatment stop. However, the effect of initial watering on growth completely vanished in the second season.

Grazing had a negative effect on *B. retusum* survival, in particular in the beginning of the study period. Grazing is known to increase early seedling mortality, thereby hampering establishment of the species (Buisson et al. 2015). Although palatability is low compared with dicots and several other grasses, young seedlings are still attractive to sheep (Buisson et al. 2015) and play an important role in sheep nutrition (Meuret et al., 2013). Apart from biomass removal by grazing sheep, trampling, urine and faeces excre-

tion may also reduce survival as already detailed in the germination section (Bilotta et al. 2007). Negative grazing effects on survival are smaller after autumnal regrowth, confirming the high tolerance of *B. retusum* to above-ground disturbance (Caturla et al. 2000; De Luis, Raventós, Cortina, González-Hidalgo, & Sánchez 2004). Additionally, negative grazing effects were mitigated by watering, resulting in a significant grazing \times watering interaction as discussed above.

Grazing significantly reduced nearly all growth parameters at the end of the study period. Reduced growth of defoliated grasses caused by the loss of photosynthetic active tissues has been widely observed (Ferraro & Oosterheld 2002; Van Staalduinen & Anten 2005). However, different compensatory growth mechanisms may limit the damage such as stimulating photosynthesis, removing old and dead tissues, changed resource allocation and tiller production (McNaughton 1983; Oosterheld & McNaughton 1988; Zhao, Chen, & Lin 2008). In our study, we observed increased tillering and corresponding plant diameter in the first season. Furthermore, negative effects of grazing on leaf length and plant height were not significant during the grazing break in winter 2017, suggesting compensatory growth as a major tolerance mechanism.

As expected, seedlings pre-grown in a growth chamber established better than field-germinated seedlings, confirming other plant re-establishment studies (Menges 2008; Godefroid et al. 2011). Pre-grown seedlings already have a well-developed root and rhizome network when transplanted to the field. Even in non-rhizomatous plant species, mortality often decreases with seedling age, reducing losses in transplants compared with field-sown seedlings (Gilbert, Harms, Hamill, & Hubbell 2001; Quintana-Ascencio, Ramírez-Marcial, González-Espinosa, & Martínez-Ic6 2004). Although significant, the magnitude of the seedling type effect on survival was relatively low (10%), thus not strongly advocating expensive pre-growth and transplanting. Differences in growth were much higher than differences in survival. In particular, plant height and diameter were greater in pre-grown seedlings even two seasons after planting. The large differences in tiller number and cumulative tiller length in the beginning became, however, much smaller at the end of the experiment, suggesting a catch-up in these growth traits. The response of pre-grown and field-germinated seedlings to the water and grazing treatments was similar, resulting in non-significant interactions.

Conclusions and implications

Initial watering had a positive effect on early seedling establishment but not on germination indicating that in Mediterranean dry grasslands, the second step of seedling recruitment is limited by water availability. However, the watering effect disappeared after two growing seasons except

for a significantly higher survival in grazed and watered plots. Grazing had a generally negative effect on field germination and seedling establishment during the first two years. In ecological restoration, initial fencing (at least two years) increases thus germination, survival and growth of introduced populations (Buisson et al. 2015). Fencing may also be sufficient to avoid water limitation and the need to irrigate grazed plots. Alternatively, watering may help to mitigate negative grazing effects such as biomass removal, mechanical damage by trampling if fencing is not possible. Seedlings pre-grown under optimum conditions established better than field-sown seedlings. However, the effect magnitude was relatively low for seedling survival, and field-sown seedling recruitment was higher than expected from previous studies (Coiffait-Gombault et al. 2012). Further observation is required to evaluate whether a long-term fitness advantage justifies the higher costs of pre-growth and planting compared with simple sowing.

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Appendix A. Supplementary data

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